

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings of claims in the application:

LISTING OF CLAIMS:

1. (ORIGINAL) A method for producing a capsular polysaccharide from an encapsulated bacterium comprising : culturing the encapsulated bacterium in a suitable culture medium at a suitable pH and temperature, while adjusting the pH of the culture medium to a constant value with a base or acid until adjustment with respectively base or acid is not possible anymore terminating the culturing just before the increase or decrease of the pH starts to slow down, preferably by cooling to below the temperature used for culturing harvesting the fermentation broth optionally, recovering the polysaccharide from the culture medium.

2. (ORIGINAL) Method according to claim 1 wherein the fermentation is terminated within about 6-14 hours after the start of the fermentation.

3. (CURRENTLY AMENDED) Method according to claim [[1 or 2]] wherein lysis is delayed by cooling to below 30°C, preferably below 25 or 20°C.

4. (ORIGINAL) Method according to claim 3 wherein the pH of the culture medium is adjusted with base to a constant value of between 6.5 and 7.5.

5. (CURRENTLY AMENDED) Method according to ~~claims 1-4~~ claim 1 wherein the culture medium is used to culture a strain of Haemophilus influenzae type b.

6. (ORIGINAL) Method for recovering a polysaccharide from a fermentation broth comprising :

- omitting the use of phenol, high-speed centrifugation, ultracentrifugation and chromatography,;
- maximally 4 precipitation steps.

7. (ORIGINAL) Method according to claim 6 wherein the recovery includes:

- mixing the polysaccharide fraction with a cationic detergent adding alcohol until a concentration which is below the concentration necessary for precipitating the polysaccharide.

8. (CURRENTLY AMENDED) Method according to claim 6 [[or 7]] comprising: using a cationic detergent to precipitate the polysaccharide or part of the contaminants from the supernatant to obtain a first polysaccharide fraction; using alcohol to precipitate the polysaccharide from the first polysaccharide fraction to obtain a second polysaccharide fraction; subjecting the second polysaccharide fraction to an

alcohol precipitation in the presence of an anionic detergent, whereby the alcohol is present in a concentration which is below the concentration at which the polysaccharide precipitates; precipitating the polysaccharide from the soluble fraction using alcohol to obtain a polysaccharide precipitate; dissolving the polysaccharide precipitate and subjecting it to concentration and diafiltration.

9. (CURRENTLY AMENDED) Method according to claim 8 wherein the polysaccharide is a capsular polysaccharide which has been produced according to the method of ~~claim 1-5~~ claim 1.

10. (CURRENTLY AMENDED) Method for producing a polysaccharide conjugate vaccine which method comprises:

- producing a polysaccharide according to the method of ~~claims 1-5~~ claim 1
- recovering the polysaccharide from the culture medium
- optionally, activating the recovered polysaccharide for conjugation - conjugating the recovered polysaccharide to a protein carrier, preferably a toxoid
- optionally, purifying the polysaccharide-protein conjugate.

11. (CURRETNLY AMENDED) Method according to claim 10 wherein the polysaccharide is recovered from the culture medium by ~~using a process according to claim 6 or 7. - omitting the use~~

of phenol, high-speed centrifugation, ultracentrifugation and chromatography,; and

- carrying out maximally 4 precipitation steps.

12. (CURRENTLY AMENDED) Method according to claim 10 [[or 11]] wherein the polysaccharide is subjected to controlled alkaline degradation in the presence of a bicarbonate/carbonate buffer under vigorous agitation before activation or conjugation.

13. (CURRENTLY AMENDED) Method according to claim 11 [[or 12]] wherein the polysaccharide is activated and then purified by using a tangential flow filtration system.

14. (CURRENTLY AMENDED) Method according to ~~claims 10-13~~ claim 10 wherein the activated polysaccharide is conjugated to protein at a pH in the range of pH 4.0 to 6.5, wherein the pH is regulated by a buffer devoid of carboxylic acid groups.

15. (ORIGINAL) Method according to claim 14 wherein the pH is regulated by a 2-morpholino ethanesulfonic acid (MES) buffer at pH 5.5 to 6.1.

16. (CURRENTLY AMENDED) Method according to ~~claims 1-15~~ claim 1 wherein the polysaccharide is polyribosyl ribitol phosphate.

17. (CURRENTLY AMENDED) Pharmaceutical composition comprising a polysaccharide or polysaccharide conjugate which is produced according to the method of ~~claims 1-16~~ claim 1.

18. (NEW) Method according to claim 2 wherein lysis is delayed by cooling to below 30°C, preferably below 25 or 20°C.

19. (NEW) Method according to claim 7 comprising: using a cationic detergent to precipitate the polysaccharide or part of the contaminants from the supernatant to obtain a first polysaccharide fraction; using alcohol to precipitate the polysaccharide from the first polysaccharide fraction to obtain a second polysaccharide fraction; subjecting the second polysaccharide fraction to an alcohol precipitation in the presence of an anionic detergent, whereby the alcohol is present in a concentration which is below the concentration at which the polysaccharide precipitates; precipitating the polysaccharide from the soluble fraction using alcohol to obtain a polysaccharide precipitate; dissolving the polysaccharide precipitate and subjecting it to concentration and diafiltration.

20. (NEW) Method according to claim 11 wherein the polysaccharide is subjected to controlled alkaline degradation in the presence of a bicarbonate/carbonate buffer under vigorous agitation before activation or conjugation.